REMARKS

Claims 1-15 are under examination; claims 16 and 17 have been withdrawn. Claim 1 has been amended above; support for the amendments can be found at least in original claim 3 and on page 7, second paragraph and last paragraph. Claim 2 has been canceled and claims 3 and 4 have been amended so that they conform with the amended language of claim 1.

Claims 1, 2 and 4-15 remain rejected under 35 U.S.C. § 102(b) as anticipated by U.S. Patent 5,506,121, issued to Skerra et al. The examiner asserted that the '121 patent discloses a "filter sandwich test" in a method for identifying a cell colony which expresses a soluble target protein, including the steps of growing cell colonies on a first filter or nitrocellulose membrane, filtering the lysate through the filter and detecting the protein which has passed through with an antibody. The examiner acknowledged that Applicants argued in their response to the last Office Action that the '121 patent does not comprise a lysis step. She asserted, however, that this argument is not persuasive as the claimed method encompasses a "native" lysis which allows capturing a soluble protein expressed by a growing colony of cells. She noted that the claims do not require the use of lysozyme as a lysing agent, that in the cited method a protein separated from the cells as a result of a

"native" lysis is diffused or filtered through the upper grow membranes to the lower capture membranes and further detected, and that the cited method thus comprises the same steps presently claimed. This rejection is traversed.

Applicants again respectfully submit that the '121 patent does not anticipate the claimed invention. Applicants respectfully submit that the examiner's interpretation of "lysis" is extremely broad and is not consistent with the discussion of lysis provided in the current specification at, for example, page 7, where it is clear that the lysis step of the present invention is an active step; i.e., the cell colonies are subjected to particular conditions which are capable of causing lysis thereof. This is specifically indicated in claim 1 and clearly represents actively exposing the cells to conditions under which the cells are capable of lysis. One of ordinary skill in the art would appreciate that normal growing conditions would not represent conditions which are capable of causing cell lysis.

Indeed, natural lysis as discussed by the examiner is an extremely rare event; if cells would spontaneously lyse it would be very difficult, for example, to carry out large scale fermentations of microorganisms. The examiner's assertion that the lysis step of the claims encompasses natural lysis which

could occur in the method of the '121 patent, therefore, is not appropriate.

In addition, the term "native" as used in claim 2 is equivalent to defining the lysis as being non-denaturing, i.e., as allowing the proteins to retain their native conformation. It does not mean that some type of native lysis is occurring. This is clearly defined at page 7 of the present application, which provides that "[p]referably the lysis step is non-denaturing, allowing proteins to retain a native, i.e. correctly folded or native-like conformation, this is referred to herein as 'native lysis.'" For purposes of advancing prosecution, however, Applicants have deleted claim 2 from the application and have amended claim 1 above to provide that the cells are subjected to conditions which are capable of causing non-denaturing lysis thereof. The '121 patent does not disclose or suggest conditions which would be capable of causing non-denaturing lysis of cells.

Applicants also wish to point out that there is further evidence that the '121 patent does not use a lysis step. The lysozyme which is coated on the second membrane in the method of the '121 patent cannot be free to travel through to the first membrane upon which the cell colonies are present, otherwise the detection step and the whole method of the '121 patent may not work. Thus, there may not be lysozyme on the second membrane to

capture the filtered secreted protein if the lysozyme was free to diffuse through to the first membrane. This would represent a very inaccurate assay if the binding target is not where it is supposed to be.

Claims 1-15 remain rejected under 35 U.S.C. §102(b) as anticipated by Knaust et al., *Anal. Biochem.* 297:79-85 (2001). The examiner asserted that the lysis step in this reference falls within the scope of the present claims and he indicates that the reference discloses a step of "subjecting a cell originating from a single cell colony to conditions which are capable of causing lysis." The examiner noted that in their response to the previous Office Action the Applicants had argued that the method of Knaust et al. encompasses the lysis of cells in liquid medium and, therefore, cell colony lysis is not contemplated. The examiner asserted that this argument is unpersuasive as the present claims do not exclude lysis of cells in a liquid medium. The examiner further noted that the Knaust et al. reference contemplates analysis of a single colony at, for example, page 82, col. 1, line 2. This rejection is traversed.

By amendment above, claim 1 now specifically provides that the lysis step is not carried out on colonies grown in liquid culture. This disclaimer language is based on page 7, second paragraph, of the specification, which specifies that

"'[c]olonies' do not encompass cells grown in liquid culture." This language excludes cell colonies grown in liquid culture but does not exclude cells grown in liquid culture prior to plating onto solid media and developing into the colony.

With regard to the examiner's comment regarding page 82 of the Knaust et al. reference, Applicants note that the colony clearly was grown in liquid LB medium and as such is not encompassed by the amended language of claim 1.

Claims 1-15 remain rejected under 35 U.S.C. §103(a) as unpatentable over the '121 patent and the Knaust reference. The examiner again asserted that the two references teach or suggest all claimed limitations and that it would have been obvious to apply either lysing buffer or freeze-thawing cells in order to lyse cells and to liberate soluble proteins with a reasonable expectation of success in filtrating and detecting soluble proteins in the method of identifying cell colonies expressing soluble target proteins as taught or suggested by the references. This rejection is traversed.

Applicants respectfully submit that the claims as amended above are both patentably distinct from, and unobvious over, the cited references. Neither the '121 Skerra et al. patent nor the Knaust et al. reference teach or suggest that lysis of cells and filtration of lysates can be carried out directly on colonies of

cells on solid or semi-solid media. The direct lysis step of the invention, which is neither taught nor suggested by the prior art, has enabled the development of a method which is capable of operating on large numbers of variants, is inexpensive and has a high reliability in predicting soluble variants of proteins. One of skill in the art would not appreciate from the teachings of the cited references that a soluble protein screen could be developed which would employ a step of direct colony lysis as set forth in the pending claims.

Furthermore, the '121 patent is not concerned with the identification of soluble protein variants and there is no suggestion that the method cited by the examiner in that reference could be used to detect soluble protein variants (or that such a method could be adapted to use a direct lysis step or any lysis step). With regard to the Knaust et al. paper, this reference teaches away from using a direct lysis step. As can be seen in Figure 2 of that document, the original colonies on solid media are actively picked and grown in liquid media and then lysed in liquid media. There is no suggestion as to how the disclosed method could be improved and certainly no indication that the original colonies could be directly lysed. One of ordinary skill in the art would not carry out such a step in view of the teachings of Knaust et al. The claimed invention has

enabled the development of a highly efficient screening tool which was not even alluded to in the prior art. The claimed invention thus is not obvious over the cited combination of references.

In view of the foregoing amendments and discussion, Applicants respectfully submit that the claims of this application are in condition for allowance.

☒ Customer Number or Bar Code Label **6449**					
Name	Barbara G. Ernst, Reg. No. 30,377				
Signature	/ Barbara G. Ernst /			*Date*	September 2, 2009
Address	Rothwell, Figg, Ernst & Manbeck Suite 800, 1425 K Street, N.W.				
City	Washington	*State*	D.C.	*Zip Code*	20005
Country	U.S.A.	*Telephone*	202-783-6040	*Fax*	202-783-6031